

1 WHAT IS CLAIMED IS:

- 2 1. A method for purifying plasmid DNA from bacterial cells, the
3 method comprising the following steps:
4 a) contacting the cells with a lysis solution, thereby forming a lysis
5 mixture;
6 b) flowing the lysis mixture through a first static mixer to obtain a
7 lysed cell solution;
8 c) contacting the lysed cell solution with a precipitation solution;
9 d) flowing the lysed cell solution and the precipitation solution through
10 a second static mixer thereby forming a precipitation mixture;
11 e) centrifuging the precipitation mixture, thereby forming a pellet and a
12 clarified solution comprising the plasmid DNA; *precipitation mixture*
13 f) neutralizing either the ~~precipitate solution~~ or the clarified solution;
14 and,
15 g) contacting the clarified solution with a positively charged ion
16 exchange chromatography resin, wherein the plasmid DNA is eluted from the ion
17 exchange chromatography resin with a saline step or continuous gradient; thereby forming
18 a purified plasmid DNA solution.

1 2. The method of claim 1, further comprising the step of RNase
2 digestion.

1 3. The method of claim 1, wherein the lysis solution contains alkali.
1 4. The method of claim 1, wherein the precipitation solution contains
2 potassium acetate.

1 5. The method of claim 1, wherein the neutralizing step precedes the
2 step of centrifuging the precipitation mixture.

1 6. The method of claim 1, wherein the linear velocity of the lysis
2 mixture through the first static mixer is between about 0.38 to 2.3 feet per second and the
3 first static mixer has an outer diameter in the range of from about 3/16" inch to about 2
4 inches.

- 1 7. The method of claim 6, wherein the first static mixer has 24
2 elements.
- 1 8. The method of claim 6, wherein the first static mixer is a laminar
2 flow static mixer.
- 1 9. The method of claim 1, wherein the linear velocity of the
2 precipitation mixture through the second static mixer is between 0.38 to 2.3 feet per
3 second and the second static mixer has an outer diameter in the range of from about 3/16
4 inch to about 2 inches.
- 1 10. The method of claim 9, wherein the second static mixer is a laminar
2 flow static mixer.
- 1 11. The method of claim 9, wherein the second static mixer has 24
2 elements.
- 1 12. The method of claim 1, wherein steps (a) and (b) are carried out
2 simultaneously.
- 1 13. The method of claim 1, wherein steps (c) and (d) are carried out
2 simultaneously.
- 1 14. The method of claim 1, wherein steps (a), (b), (c), and (d) are
2 carried out simultaneously. *[Handwritten mark: a large circle with a diagonal line through it, with the number 1 written above it and the number 2 written below it.]*
- 1 15. The method of claim 1, wherein steps (a), (b), (c), (d) and (e) are
2 carried out simultaneously.
- 1 16. The method of claim 1, wherein steps (a), (b), (c), (d) (e) and (f) are
2 carried out simultaneously. *[Handwritten mark: a large circle with a diagonal line through it, with the number 1 written above it and the number 2 written below it.]*
- 1 17. The method of claim 16, wherein the method is automated.

1 *Subj 2* 18. The method of claim 1, further comprising filtering the supernatant
2 through an ultrafiltration unit comprising a gel layer before contacting the supernatant with
3 the positively charged ion exchange resin.

1 19. The method of claim 18, wherein the ultrafiltration unit comprises a
2 membrane having a molecular weight cutoff of from about 50K to about 500K daltons.

1 20. The method of claim 1, further comprising ultrafiltration of the
2 plasmid DNA using tangential flow ultrafiltration with an open channel device, in the
3 presence of a gel layer.

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*lack of specific
antecedent basis
in cl. 1*